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Molecular analysis of NDM-1-producing enterobacterial isolates from Geneva, Switzerland

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Objectives: To analyse the mechanisms responsible for decreased susceptibility or resistance to carbapenems in several enterobacterial isolates recovered in 2009–10 in Geneva University Hospitals, Switzerland.

Methods: PCR and sequencing were used to identify β-lactamases, 16S RNA methylases and plasmid-mediated quinolone resistance genes. The transferable properties of the plasmids were analysed, as well as their plasmid type. The strains were typed by multilocus sequence typing.

Results: Three patients were found to be positive for NDM-1-producing enterobacterial isolates (one with *Escherichia coli* and *Klebsiella pneumoniae*, one with *K. pneumoniae* only and one with *Proteus mirabilis*), where NDM-1 stands for New Delhi metallo-β-lactamase-1. The bla_{NDM-1} carbapenemase gene was detected in all isolates in addition to genes encoding narrow-spectrum β-lactamases (TEM-1, SHV-11, OXA-1, OXA-9 and OXA-10), extended-spectrum β-lactamases (CTX-M-15, CMY-16 and CMY-30), ArmA and quinolone resistance determinants (Qnr). The bla_{NDM-1} gene was located on conjugative IncA/C- or IncF-type plasmids. Upstream of the bla_{NDM-1} gene, part of IS*Aba125*, previously identified in NDM-1-negative *Acinetobacter baumannii*, was found. Downstream of the bla_{NDM-1} gene, variable sequences were found.

Conclusions: This work constitutes the first identification of NDM-1 producers in Switzerland. Interestingly, patients from whom these NDM-1-producing isolates were recovered had a link with the Indian subcontinent or the Balkans.

Keywords: metallo-β-lactamases, carbapenems, Enterobacteriaceae

Introduction

The New Delhi metallo-β-lactamase-1 (NDM-1) is an Ambler class B β-lactamase that confers resistance to all β-lactams except aztreonam.[1] Initially reported from Sweden in *Klebsiella pneumoniae* and *Escherichia coli* from a patient transferred from India,[2] NDM-1-producing isolates have been particularly associated with the UK, India and Pakistan;[3] although, more recently, such isolates as well as other species, including *Acinetobacter baumannii*, have been reported from other parts of the world.[1] Recent data have suggested that the Balkans and the Middle East might serve as secondary reservoirs of NDM-1.[4–9] Here, we report a retrospective study of enterobacteria exhibiting decreased carbapenem susceptibility that were isolated in Geneva University Hospitals from March 2009 to October 2010. Four isolates that were found to produce NDM-1 were subsequently subjected to detailed molecular analysis.

Materials and methods

Bacterial isolates and susceptibility testing

The enterobacterial isolates were identified by using a MicroFlex MALDI-TOF/MS instrument using Biotyper 2 software (Bruker Daltonics, Fällanden, Switzerland). The antibiotic susceptibilities of the isolates and their transconjugants were determined first by the disc diffusion technique on Mueller–Hinton agar plates with β-lactam and non-β-lactam antibiotic-containing discs, and interpreted according to CLSI guidelines.[10] Then, precise MIC values were determined by using Etest strips (AB bioMérieux, Solna, Sweden). Azide-resistant *E. coli* J53 (Invitrogen, Cergy-Pontoise, France) was used as the host in conjugation experiments.[11]

PCR amplification and sequencing

Multiplex PCR approaches were used to detect different types of β-lactamase genes,[12,13] plasmid-mediated aminoglycoside resistance

genes[14] and plasmid-mediated quinolone resistance genes.[15] PCR experiments were performed to identify the upstream- and downstream-located regions of the bla_{NDM-1} gene.[11] All amplified DNA fragments were purified using the Qiaquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced using an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available over the Internet at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

Plasmid analysis

Conjugation assays were performed between clinical isolates as donors and an azide-resistant *E. coli* J53 as the recipient strain, using a selection based on ceftazidime (30 mg/L) and azide (100 mg/L).[11] Plasmid DNAs were extracted using the method of Kieser.[16] Then, the plasmid incompatibility groups were determined by a PCR-based replicon typing method, as described previously.[17]

Strain genotyping

Multilocus sequence typing (MLST) methods were used as previously described for *E. coli* and *K. pneumoniae* isolates.[18,19]

Results and discussion

Twenty enterobacterial isolates with decreased susceptibility to ertapenem were isolated at Geneva University Hospitals from March 2009 to October 2010. Among them, four isolates were found to be positive for bla_{NDM-1} by a preliminary PCR-based analysis. They were a single *E. coli* (strain 5649), two *K. pneumoniae* (strains 6642 and 6759) and a *Proteus mirabilis* (strain 7892). *E. coli* 5649 and *P. mirabilis* 7892 were from rectal flora (carriage), and *K. pneumoniae* 6642 and 6759 were from urinary tract infections. *E. coli* 5649 and *K. pneumoniae* 6759 were from the same Serbian patient, *K. pneumoniae* 6642 was from a patient previously hospitalized in India, and *P. mirabilis* 7892 was from a patient of Pakistani origin. These four enterobacterial isolates had either decreased susceptibility or were fully resistant to the four carbapenems tested (Table 1). Of note, NDM-1 production was not always associated with higher MICs of ertapenem or imipenem (Table 1). A combined disc test performed using 930 μg of EDTA and a disc of imipenem with all the isolates as template gave positive results, suggesting metallo-β-lactamase production. In addition, those isolates were overall resistant to most antibiotics, including aminoglycosides, fluoroquinolones, sulphonamides, trimethoprim and rifampicin, with a few exceptions.

K. pneumoniae 6642 remained susceptible to tetracycline, tigecycline, fosfomycin and colistin, *K. pneumoniae* 6759 was susceptible to tigecycline, fosfomycin, colistin, ciprofloxacin and amikacin, *E. coli* 5649 was susceptible to tigecycline, fosfomycin, colistin, chloramphenicol and nitrofurantoin, and *P. mirabilis* 7892 was susceptible to tigecycline, fosfomycin, fluoroquinolones, trimethoprim and rifampicin.

As opposed to the findings of previous studies identifying NDM-1 producers, no other carbapenemase genes (OXA-48 or VIM types) were identified in these isolates.[20] Downstream of the bla_{NDM-1} gene, a putative bleomycin resistance gene was identified in strain *E. coli* 5649. However, it was not detected in the other isolates. Upstream of the bla_{NDM-1} gene, part of IS*Aba125* was identified in all isolates. This IS*Aba125* element has been previously identified in NDM-1-negative *A. baumannii* isolates.[21] This result further indicated that part of the IS*Aba125* sequence is constantly present upstream of the bla_{NDM-1} gene in Enterobacteriaceae. It is therefore likely that this insertion sequence (IS) element may have played a role in the mobilization of bla_{NDM-1}, at least in the early processes of its acquisition. It also suggests that the original dissemination of the bla_{NDM-1} gene occurred in *A. baumannii* and that its spread among Enterobacteriaceae might correspond to a secondary event.

Transconjugants were obtained from all clinical strains, indicating that the bla_{NDM-1} gene was always located on conjugative plasmids. Two different plasmid scaffolds carrying bla_{NDM-1} were identified, being IncF and IncA/C (Table 2). MICs of carbapenems for those transconjugants were much lower than those obtained for clinical strains, indicating that multiple carbapenem resistance mechanisms are probably present in those clinical isolates (Table 1). In addition to bla_{NDM-1}, genes encoding TEM-1, OXA-1, OXA-10 and SHV-11, the plasmid-mediated cephalosporinases

Table 1. MICs (mg/L) of carbapenems and other antibiotics for clinical isolates *K. pneumoniae* 6642 and 6759, *E. coli* 5649 and *P. mirabilis* 7892, their transconjugants in *E. coli* J53 and *E. coli* J53

Strain	IPM	ETP	MEM	DOR	GEN	AMK	Sulphonamides	RIF	TMP	CIP
K. pneumoniae 6642	1	16	4	2	>32	>32	>32	>32	>32	>32
Tc6642	1	1	1	1	>32	>32	>32	>32	>32	0.5
K. pneumoniae 6759	8	>32	32	>32	16	4	>32	>32	>32	0.5
Tc6759	1	0.5	0.5	0.25	8	0.5	>32	>32	32	0.5
E. coli 5649	8	>32	16	8	>32	>32	>32	8	>32	>32
Tc5649	2	0.5	1	1	>32	>32	>32	4	>32	0.06
P. mirabilis 7892	2	0.5	0.5	0.5	>32	>32	>32	8	8	0.12
Tc7892	1	0.5	0.5	0.25	>32	>32	>32	4	0.25	0.06
E. coli J53	0.06	0.06	0.06	0.06	0.06	0.12	0.25	2	0.25	0.06

Tc, transconjugant; IPM, imipenem; ETP, ertapenem; MEM, meropenem; DOR, doripenem; GEN, gentamicin; AMK, amikacin; RIF, rifampicin; TMP, trimethoprim; CIP, ciprofloxacin.

Table 2. Features associated with the bla_{NDM-1} plasmids

Strain	Plasmid type carrying bla_{NDM-1}	Size (kb)	Associated resistance determinants[a]
E. coli 5649	IncF	130	TEM-1, OXA-1, CMY-30, ArmA
K. pneumoniae 6642	IncA/C	150	CTX-M-15, TEM-1, SHV-28, OXA-1, RmtA, QnrB1
K. pneumoniae 6759	IncA/C	150	CTX-M-15, TEM-1, SHV-11, OXA-1, OXA-9, OXA-10, CMY-16, QnrA6
P. mirabilis 7892	IncA/C	150	TEM-1, OXA-1, OXA-10, CMY-16, ArmA

[a]Underlined resistance markers are those encoded by the bla_{NDM-1} plasmid and therefore identified in the respective *E. coli* transconjugants.

OXA-9, CMY-16 and CMY-30 (derivatives of CMY-2), the extended-spectrum β-lactamase CTX-M-15, SHV-28, the quinolone resistance proteins QnrA6 and QnrB1, and the 16S rRNA methylase ArmA and RmtA were identified (Table 2). Plasmids carrying bla_{NDM-1} varied in size, being either of 130 or 150 kb. Noteworthy, the three IncA/C-type plasmids were of the same size, but they differed by the resistance genes they harboured (Table 2). The identification of the bla_{NDM-1} gene on IncA/C- and IncF-type plasmids has been reported previously.[3,22]

MLST showed that *K. pneumoniae* 6642 and 6759 strains belonged to two different sequence types (STs), namely ST147 and ST25. Interestingly, an ST147-type NDM-1-positive *K. pneumoniae* has been recently identified from Iraq.[4] *E. coli* 5649 belonged to ST410, previously identified in Norway for an NDM-1-positive *E. coli* isolate recovered from a patient who had been hospitalized in India.[23]

This study constitutes the first report of the identification of NDM-1 producers in Switzerland. Only a single enterobacterial isolate producing a carbapenemase has been reported from Switzerland so far, corresponding to a KPC-2-producing *K. pneumoniae*.[24] Since Switzerland is one of the most open countries to travellers, it is likely that the spread of such carbapenemase producers may be increasingly identified in the near future and a detection strategy should be implemented there. The origin of one of the patients (Serbia) further underlines that the Balkan countries might be an additional source of NDM-1 producers.[8] The studied isolates were multidrug resistant, and expressed clavulanic acid-inhibited extended-spectrum β-lactamases, plasmid-mediated cephalosporinases and wide-spectrum aminoglycoside resistance enzymes, further underlining the wide-spectrum activity of all resistance determinants gathered among NDM-1 producers. Plasmids carrying the bla_{NDM-1} gene did not co-harbour as many other resistances as seen with other reported plasmids encoding NDM-1. This suggests that the process of multidrug resistance in NDM-1 producers is the result of successive acquisitions of resistance genes rather than the acquisition of a single bla_{NDM-1}-positive plasmid carrying many resistance genes.

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Transparency declarations

None to declare.

References

1 Nordmann P, Poirel L, Toleman MA *et al*. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? *J Antimicrob Chemother* 2011; **66**: 689–92.

2 Yong D, Toleman MA, Giske CG *et al*. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009; **53**: 5046–54.

3 Kumarasamy KK, Toleman MA, Walsh TR *et al*. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; **10**: 597–602.

4 Poirel L, Fortineau N, Nordmann P. International transfer of NDM-1-producing *Klebsiella pneumoniae* from Iraq to France. *Antimicrob Agents Chemother* 2011; **66**: 304–6.

5 Poirel L, Al Maskari Z, Al Rashdi F *et al*. NDM-1-producing *Klebsiella pneumoniae* isolated in the Sultanate of Oman. *J Antimicrob Chemother* 2011; **66**: 304–6.

6 Zarfel G, Hoenigl M, Leitner E *et al*. Emergence of New Delhi metallo-β-lactamase, Austria. *Emerg Infect Dis* 2011; **17**: 129–30.

7 Bogaerts P, Verroken A, Jans B *et al*. Global spread of New Delhi metallo-β-lactamase 1. *Lancet Infect Dis* 2010; **10**: 831–2.

8 Livermore DM, Walsh TR, Toleman M *et al*. Balkan NDM-1; escape or transplant? *Lancet Infect Dis* 2011; **11**: 164.

9 Leverstein-van Hall MA, Stuart JC, Voets GM *et al*. Global spread of New Delhi metallo-β-lactamase 1. *Lancet Infect Dis* 2010; **10**: 830–1.

10 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20U*. CLSI, Wayne, PA, USA, 2010.

11 Poirel L, Lagrutta E, Taylor P *et al*. Emergence of metallo-β-lactamase NDM-1-producing multidrug-resistant *Escherichia coli* in Australia. *Antimicrob Agents Chemother* 2010; **54**: 4914–6.

12 Poirel L, Walsh TR, Cuvillier V *et al*. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; doi:10.1016/j.diagmicrobio/2010.12.002.

13 Poirel L, Girlich D, Naas T *et al*. OXA-28, an extended-spectrum variant of OXA-10 β-lactamase from *Pseudomonas aeruginosa* and its plasmid- and integron-located gene. *Antimicrob Agents Chemother* 2001; **45**: 447–53.

14 Berçot B, Poirel L, Nordmann P. Plasmid-mediated 16S rRNA methylases among extended-spectrum β-lactamase-producing Enterobacteriaceae isolates. *Antimicrob Agents Chemother* 2008; **52**: 4526–7.

15 Cattoir V, Poirel L, Rotimi V *et al*. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007; **60**: 394–7.

16 Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* 1984; **12**: 19–36.

17 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

18 Tartof SY, Solberg OD, Manges AR *et al*. Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. *J Clin Microbiol* 2005; **43**: 5860–4.

19 Diancourt L, Passet V, Verhoef J *et al*. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82.

20 Poirel L, Carricajo A, Berthelot P *et al*. Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. *Antimicrob Agents Chemother* 2010; **55**: 447–8.

21 Evans BA, Hamouda A, Towner KJ *et al*. Novel genetic context of multiple bla_{OXA-58} genes in *Acinetobacter* genospecies 3. *J Antimicrob Chemother* 2010; **65**: 1586–8.

22 Poirel L, Revathi G, Bernabeu S *et al*. Detection of NDM-1-producing *Klebsiella pneumoniae* in Kenya. *Antimicrob Agents Chemother* 2011; **55**: 934–6.

23 Samuelsen O, Thilesen CM, Heggelund L *et al*. Identification of NDM-1-producing Enterobacteriaceae in Norway. *J Antimicrob Chemother* 2011; **66**: 670–2.

24 Poirel L, Lienhard R, Potron A *et al*. Plasmid-mediated carbapenem-hydrolysing β-lactamase KPC-2 in a *Klebsiella pneumoniae* isolate from Switzerland. *J Antimicrob Chemother* 2011; **66**: 675–6.